THE PHYSIOLOGICAL EFFECTS OF SELENIUM COMPOUNDS WITH RELATION TO THEIR ACTION ON GLYCOGEN AND SUGAR DERIVATIVES IN THE TISSUES

By CHARLES O. JONES, M.D. (Liverpool).

Communicated by Prof. Benjamin Moore

From the Bio-Chemical and Physiological Departments, University of Liverpool

HISTORICAL.

Action on Plants and Bacteria.—The earliest studies on plants and bacteria were made by Knop in 1885. He found that on adding traces of selenium and tellurium salts to the water in which plants grew, that although no influence on the growth of the plants took place, yet selenium was absorbed. The same was found true of algae and infusoria by Bokorny in 1893. Scheurlen, in 1900, seeking a substance which contained loosely-bound oxygen, to grow bacteria in absence of atmospheric oxygen, tried sodium selenite, and found that though the bacteria were unaffected, yet they were coloured with the reduced selenium. This selenium was found entirely in the cell, none being found in the media. A careful study of these effects was conducted by Klett, who found that bacteria and moulds were not as a rule hindered in their development by traces of selenite of sodium, but a few, such as the bacillus of malignant oedema and symptomatic anthrax, were arrested in growth. He also found the bacteria coloured with the reduced selenium, the surrounding media being colourless, and that as the amount of selenite was increased growth was inhibited. He concluded that the reduction of selenite to selenium took place in the protoplasm of the bacterial cell, and not outside the cell by secondary action of metabolic products.

Action on animals.—Gmelin appears to be the first who investigated the effects of selenium and tellurium salts on animals. He found that they were poisonous, that they produced a deposit of the reduced element on the intestinal walls, and that the animal gave off a garlic-like odour. This odour was also noticed in the animal's breath by Hansen, who attributed it to ethyl selenide; this observer found also that after a large

dose the animal vomited, and that the vomit contained selenium. On making sections of the animal's organs he noticed that they all contained selenium deposited in granules. The work was continued by Rabuteau, who observed that after a large dose vomiting, profound dyspnoea, anasthesia, opesthotonos, and death from asphyxia took place. The post-mortem findings were intense congestion and ecchymosis of the whole intestinal tract, also of the liver, spleen, lungs, and kidney. The right side of the heart and large blood vessels held a multitude of small prismatic crystals of unknown chemical composition. Rabuteau concluded that these crystals acted as a mechanical obstruction and caused death. These results were not confirmed by Czapek and Weil, who could not find any crystals or mechanical obstruction, and concluded that selenium was very similar in its action to tellurium, arsenic and antimony, and that death was due to paralysis of the so-called excito-motor ganglia. They further noticed marked distension of the abdominal capillaries. The blood was normal, but it gave off a marked garlic-like odour. This odour was noticed by Wöhler to be similar to methyl selenide, which he was then preparing. Hofmeister confirmed this, proving by analysis that they were the same, and further showed that all the organs gave off the odour, but that it was most pronounced in the testes and lungs, and marked in the blood, liver and kidney. If the organs were placed in an incubator the smell was intensified, but blood loses the smell. Hofmeister concluded that all the organs could absorb selenium and form methyl selenide from it; lastly he discovered that the reduction to selenium and the formation om methyl selenide were independent of one another. On heating an organ to 55° C. the formation of methyl selenide ceases, but the organ will still reduce selenite to selenium. The explanation suggested was that the reduced selenium was slowly built up into a soluble compound in the alkaline blood and was changed in the lungs to methyl selenide. The methyl groups he supposed to be derived from cholin, creatinin, and other methyl-bearing substances. The effects of selenium and tellurium salts on metabolism were investigated by Mead and Gies, and Woodruff and Gies, who found that selenium salts had little or no effect on metabolism, but that the ether-soluble substance in the faeces was increased. This they attributed to diminished absorption. They also examined the vomit resulting from selenium salts, and found that there was complete absence of free hydrochloric acid, the pepsin was unaltered, and on addition of hydrochloric acid digestion proceeded at a normal rate. Ptyalin, on the other hand, was markedly affected by selenium salts,

The compounds of selenium are thus extremely toxic; but if the amount present is very small, the cells are able to reduce it, forming an inert substance, and can then continue their metabolic changes.

This research was undertaken to endeavour to find out how this reduction is accomplished and also to find out more exactly the cause of death in selenium poisoning.

Two compounds of selenium were used in this reasearch, viz., sodium selenite and sodium selenate, both obtained from Kahlbaum.

Sodium selenate is a comparatively stable salt, easily soluble in water and neutral in reaction.

The lethal dose of selenate for a moderate sized rat (about 80 grammes) was found to be 0·6 c.c. of 0·125 per cent. solution.

Selenite of sodium is an extremely unstable salt. It is stated by Mead and Gies to be reduced by all protoplasm, and they have seen reduction take place in contact with fresh meat. It is even said that reduction takes place in contact with all organic matter. If this is so, it is difficult to see how any can be absorbed if given by the mouth, so that in this research all doses were given hypodermically.

The preparation used was found to be acid in reaction, the acidity of 1 gramme being equal to 2·75 c.c. normal sulphuric acid. It is distinguished from selenate most easily by the insolubility of the selenites of copper, cobalt and nickel. Cobalt salts give a mauve precipitate visible 1 in 800 of water; copper salts give an apple-green precipitate visible 1 in 1,200 water; nickel salts give a green precipitate visible 1 in 1,600 water. Owing to its instability the selenite solution was always made fresh as required.

The lethal dose for a moderate sized rat was 0·4 c.c. of 0·125 per cent. solution. The lethal dose for a moderate sized rabbit was 0·5 c.c. of 2 per cent. solution. The lethal dose for a moderate sized cat was 1 c.c. of 2 per cent. solution.

From these results it appears that selenate of sodium is only two-thirds as toxic as selenite.

After a *small* dose no symptoms were observed, and even the appetite was unaffected. As the dose was increased and was just sub-lethal, it was observed that, after about ten minutes, the animal became restless; this was followed by movements of the mouth, tongue and nose. As there were now present a peculiar garlic-like odour in the breath, it is probable that these movements are due to stimulation of the nerves

of taste and smell. Very shortly afterwards retching and vomiting commenced. If the dose has not been too great, recovery soon takes place, no after effects being noticeable. If the dose be too.great, the vomiting and retching continue and somnolence passes on to unconsciousness and death.

It may be mentioned, as death has been ascribed by previous workers to dyspnoea, that laboured breathing was seen in one case only. It was in this case due to excessive reduction of the salt to selenium and consequent embolism of the pulmonary vessels. The paralysis, convulsions and other symptoms noticed by former investigators were probably due to the same cause, and are in no way connected with death from chemical poisoning with selenium salts.

MACROSCOPIC AND MICROSCOPIC CHANGES IN THE TISSUES

The macroscopic *post-mortem* changes were very few. The liver was usually soft and friable. All the organs gave off the garlic-like odour noticed in the breath. The right side of the heart was distended and full of clot. The splanchnic vessels were enormously dilated.

The microscopic changes were more pronounced, and were investigated as follows:—The tissues were immediately placed in formol, dehydrated with acetone, embedded in paraffin and stained with eosin and haematoxylin.

The most noticeable feature of all the sections was a golden-brown amorphous deposit found in almost every organ. It is chiefly found around the blood vessels and between the cells, but some is also to be seen inside the cells. It was suggested that this substance might be iron, but it gave none of the staining reactions for iron. On grinding up the organs with sand this substance could be extracted, and formed a brick-red deposit. This was found to be identical in every way with the amorphous form of selenium produced on reducing sodium selenite in the test-tube. It volatilized with heat, burning with a blue flame, and gave off the well-known horseradish smell of selenium.

This golden-brown deposit was found, if the dose was large, in almost every organ, the whole of the tissues being flooded with it, but this is not the cause of death, for if a just lethal dose is given there is no such flooding and death still takes place. Physiologically this deposit is inert, for if a small dose of a selenium salt is given and the animal killed some time afterwards, this deposit will be seen in the cells, which are evidently still capable in its presence of performing their metabolic changes without noticeable change.

The action of selenium salts on different isolated physiological systems prepared by the usual methods was next investigated, and they were found to be without action on (1) a muscle-nerve preparation, (2) isolated heart muscle, (3) nervous mechanism of heart, (4) higher nerve centres, (5) blood pressure, (6) intestinal movements. The urine was also normal, traces of selenium salts were found present, but never any solution that reduced Fehling's solution. The blood was occasionally found altered; the most frequent change was a slight lymphocytosis followed by a more marked increase in the polymorphonuclear leucocytes.

The red blood cells were found normal both in their number and in their haemoglobin contents, and spectroscopically the blood was found normal. In small amounts, selenate had no effect on gastric digestion, nor had it on pancreatic, while selenite had an inhibiting effect on pancreatic action, this being in part at least due to its acid reaction.

The reduction which takes place in the tissues was next investigated. After heating an organ to 60° C., as Hofmeister showed, it will still reduce selenite to selenium. This was repeated, and it was further seen that higher temperatures do not stop this reduction. It therefore seemed probable that this effect was not due to an enzyme, but was some direct chemical effect. A fresh solution of sodium selenite was therefore made, and 5 c.c. added to 0·5 gramme of each of the following carbohydrates with aseptic precautions. The mixtures were then placed in an incubator at 35° C. for twenty-four hours and again examined.

Reduction was shown by the solution becoming pale brown, while if the reduction were intense a fine brick-red powder became deposited.

POLYSACCHARIDES	Glycogen ...	No action
	Inulin ...	Profuse reduction
	Starch ...	No action
	Dextrin ...	No action
HEXATOMIC ALCOHOLS ...	Mannite ...	No action
	Dulcite ...	No action
PENTOSES	Arabinose ...	Reduction
	Rhamnose ...	No action
	Xylose ...	No action
HEXOSES	Maltose ...	Faint reduction
	Glucose ...	Reduction
	Galactose ...	Slight reduction
	Lactose ...	Slight reduction
	Levulose ...	Profuse reduction
AMINO HEXOSE	Glucosamine ...	Profuse reduction
COMPOUND SUGARS... ...	Saccharose ...	No action
	Raffinose ...	No action

The reduction of inulin is due to the formation of levulose.

It will be seen that reduction takes place with arabinose, levulose, glucose, and the sugars yielding glucose. The reduction by glucose and levulose was then tried at a low temperature, viz., 30° C., and it was found that glucose caused no reduction at this temperature even after several weeks, while levulose caused a profuse reduction. When the two sugars were mixed it was found that no reduction occurred at 30° C., and both came down at 38° C. It was thought that perhaps this reaction with more rapid heating might serve to differentiate the sugars, but on heating a solution of the sugars with sodium selenite solution in a test-tube, it was found that levulose commenced to reduce at 58° C., while glucose, lactose, galactose and maltose all reduced at about the same temperature, viz., 72° C.

The derivatives of glucose and its compound sugars were next tested as reducing agents for selenite, with the following results:—

Glucose	...	Reduction
Gluconic Acid	...	No action
Glucuronic acid	...	No action
Saccharic acid	...	Reduction
Mucic acid	...	No action
Furfurol	...	No action.

The above reductions at first sight would appear due to the aldehyde or ketone groups in the sugars, but here we are met with the fact that while rhamnose and xylose, which contain aldehyde groups, have no action, yet saccharic acid, which has neither aldehyde nor ketone group, acts very strongly. We tried the action of benzaldehyde, formaldehyde and acetone, but found they all gave negative results.

Other possible degradation products of the sugars were tried, for example, lactic acid and acetic acid were without action, but formic acid had a powerful reducing action.

It is interesting to note here that selenite is reduced by arabinose, levulose, glucose, maltose and lactose, all of which are found in, or are excreted from, the human body. The only exception is xylose, which is a necessary constituent of the nucleoproteids, but in such a position an energetic sugar would be a source of danger to the animal. These results cannot be explained on the structural formulae at present ascribed to the different sugars, so that the reduction rests more on a physiological basis than a purely chemical one. It was next ascertained whether proteins, fats, or other substances of animal origin, would perform the same

reduction. Although treated in the same way as the carbohydrate or heated together directly in the test-tube, no action was found associated with any one of them. The following substances were all tried, viz.:— Olein, oleic acid, palmitin, palmitic acid, potassium palmitate, erucic acid, lecithin, cholesterin, glycerine, uric acid, hippuric acid, urea, gelatin, glycocoll, tyrosin, casein and creatinin.

From this it appears probable that this reduction can be performed by glucose and a few allied sugars, and cannot be produced by organic matter from which carbohydrates are absent.

To ascertain whether glucose is the agent which accomplishes this reduction in animal organs, the following method was adopted. Ten grammes of finely minced liver were taken with aseptic precautions, and to this were added 5 grammes of yeast, 50 c.c. of distilled water and a few drops of toluol. A control was prepared in the same way, *but without any yeast.* Both vessels were placed in an incubator for twenty-four hours, they were then heated to 70° C. to destroy the yeast and glycolytic enzyme, and 1 gramme of sodium selenite added to each. After being in an incubator at 38° C. for a few hours, both were examined. The control which contained glucose from the glycogen was of a deep red colour, showing that quantities of selenium were present. The flask which had its sugar destroyed by the yeast showed no red colour, so that no selenite had been reduced. This experiment makes it probable that in the absence of glucose selenite is not reduced, and, accordingly, that reduction of selenite to selenium in a cell indicates the presence of glucose.

Since selenite of sodium is reduced in the cells by glucose, it appeared necessary to ascertain where, especially in the body, this reduction takes place. As has been already seen, if a large dose is given the whole organism is flooded with the selenium. A rat was therefore given several *small* doses, and was then killed, and the organs quickly removed and examined histologically.

The spleen was found to contain abundance of selenium, as well as an excessive number of leucocytes.

The portal vein was examined and found to also contain selenium and leucocytes, and the same was found true of the liver; while the vessels leaving the liver, the lungs, kidney and intestine, were found to be quite free from selenium.

In some cases the liver cells show destructive changes. The nucleus

stains less deeply while in others the nucleus has disappeared, the cells being mere shadows.

It therefore seems probable that reduction takes place firstly in the spleen. The reduced selenium is brought by the blood stream, only a small amount by leucocytes to the liver. The liver also reduces any selenite that has escaped the spleen. If the dose is not excessive no selenium is allowed to pass the liver.

DISAPPEARANCE OF GLYCOGEN FROM THE LIVER

Finding that selenite is reduced by glucose in the liver and spleen suggests that the glucose must be derived from the liver glycogen, and this was found to be the case. Two well-fed rats were taken, and to one was given an injection of sodium selenite just sublethal. As soon as it began to recover, which happened in a few hours, another injection was given. Treated in this way, in a time varying from three to seven days, the animal dies. The control rat was then killed, and the livers from the two animals contrasted as to their glycogen content by the following method:—The livers from both rats were quickly removed, cut in pieces in each case, and placed separately in boiling water acidified with acetic acid. The pieces were then ground up in each case in a mortar with hot distilled water.

In the rat which had been injected with selenite, there resulted a perfectly clear pale orange coloured solution, which gave no coloration with iodine and no precipitate with alcohol or basic lead acetate.

The fluid from the normal rat's liver gave an opalescent solution, which on addition of iodine gave a dense brown coloration.

This experiment, on account of its importance, was repeated several times, with the same result. It may be stated that the dose given must be carefully regulated so as to be just sublethal. If an over-dose is given, the animal will die, due probably to not sufficient glucose being available to reduce the selenite; glycogen will then not have disappeared entirely. This disappearance of glycogen was also found to occur in both frogs and rabbits.

This gradual using up of glycogen and glucose made it interesting to find out if any other metabolic changes took place at the same time.

A well-nourished cat was used for the experiment. The normal excretions were examined and estimated daily for a week, during which period the urine was invariably found to be acid. A small injection was

then made of 0·5 c.c. of 0·25 per cent. solution of selenite; the only change following this small dose that took place was that the urine next day had become alkaline; this continued so throughout the experiment. The dose was gradually increased, and it was noticed that the amount of urea excreted on the day following the injection had fallen considerably, but had returned to normal on the following day. As the dose increased, the urea decrement became greater, and an increased number of days were required for the return to the normal. When the dose became excessive the animal vomited. No urine was excreted the following day. The next day the excretion of urea was exceedingly small, and continued small for some days, only very slowly returning to the normal. The total amount of nitrogen excreted showed no relation to the urea; it kept steady all through the periods when the urea fell. When an excessive dose was reached the total nitrogen fell to about one-third the normal amount and gradually returned to normal.

It would thus appear that while the urea was excreted in less amount the nitrogen was got rid of in some other form. That it was not excreted as uric acid nor as ammonia seems probable as these showed little or no change throughout the experiment. Sulphates and phosphates showed no change.

The most striking effect was noticed in the excretion of chlorides. Until the dose had become excessive the amount of chlorides thrown out had continued steady. After the animal vomited the excretion of chlorides suddenly dropped, so that the daily amount excreted became half or less than half the normal amount; thus, in the first cat the average daily output was 1·28 grammes; this fell to 0·061 grammes.

In the second cat, as will be seen in the following table, the average daily excretion of chlorides for fifteen days during which it was having increasing amounts of selenite was 0·0749 grammes. After 0·75 c.c. of 2 per cent. solution had been injected the animal vomited. No urine was passed the following day. The next day the amount was only 0·0305 grammes, that is, less than half the normal amount. The following three days there was very little increase. The following day, as will be seen in the table, the whole of the retained chlorides were thrown out, the amount excreted afterwards returning to normal again.

Chlorides excreted per diem in grams.	Injection							
0·0749		Vomited	0·0305	0·0335	0·0381	0·0441	**0·1820**	0·0811
		No urine						

The vomiting took place about ten minutes after injection. The vomit consisted of the stomach contents in a state which showed that there had been no interference with digestion until the injection was given. The animal during this period was under the influence of the drug, and it is only when it reaches an excessive amount that vomiting occurs. This vomit was found, as already pointed out by Mead and Gies, to be free from any trace of free hydrochloric acid. The reaction was acid, and quantities of organic acid were present.

It therefore seems probable that the hydrochloric acid is suddenly withdrawn from the stomach to serve some other necessary purpose, and if 0·2 per cent. hydrochloric acid be added to the stomach contents digestion will proceed normally.

This withdrawal of hydrochloric acid is accompanied by a greatly diminished excretion of chlorides in the urine. This observation coincides with the withdrawal of hydrochloric acid from the stomach. While the chlorides are retained, appetite and digestion are in abeyance: after a certain period, varying from one day to five days, during which time the excretion of chlorides is only about half the normal amount, the whole of the chlorides retained are thrown out, their purpose having been fulfilled. Then the animal regains its appetite and the excretion of chlorides becomes normal.

Still another interesting fact bearing on the subject was noted : when the animal had lost its appetite, although it refused fresh meat it would still eat salt meat. Possibly the excess of chloride helped the return of hydrochloric acid.

Lastly, with these changes there was also a remarkable loss of weight. One cat lost 38 per cent. of its total weight, the average daily loss being 26 grammes. A second cat lost 335 grammes during the first week, or 16 per cent. of its weight, and in one day it even lost as much as 65 grammes. This loss of weight is too great to be accounted for by diminished consumption of food alone, for until the dosage became large the animal still retained its appetite, and even after a large dose it only refused its food for a day or two, the appetite gradually returning.

It was observed by Mead and Gies that after a dose of a selenium compound the amount of ether-soluble substance in the faeces was increased. This we found true for small doses, but on investigating the effect of large doses we found that the amount of ether-soluble matter in the faeces was very much diminished, and that the relative amounts of neutral fat, fatty acid and soap (reckoned as oleic acid) were altered.

The faeces were dried and extracted with ether in a Soxhlet apparatus, the acidity of the fat being titrated with 0·1 N alcoholic potash, using phenol phthalein as indicator. After an injection of selenite of sodium the total amount of fat in the faeces became very much reduced and was at its minimum the second day after injection. The excretion of fatty acid was less affected or increased in amount, so that the normal proportion of neutral fat to fatty acid being 2 to 1 on the second day, the fatty acid became equal to, and sometimes greater than, the amount of neutral fat. This may be seen from the following table, where the amount of fat has been worked out to a constant : - -

Fat		Free fatty acid
0·5	...	0·284
0·5	...	0·292
0·5	...	0·290
	Injection of selenite	
0·5	...	0·350
0·5	...	0·530
0·5	...	0·170
0·5	...	0·280

The effect on soaps is somewhat similar to the fatty acid, the excretion being greatest the second day after an injection and gradually falling from then onward.

These observations seem to show that glucose, or a derivative, is the means by which the body protects itself from the toxic effects of selenium salts, and death may even in certain cases be due indirectly to the using up of the glucose; but this cannot be the cause of death after a single large dose, therefore the effect of selenite on the living cells of the liver was next investigated. A fresh liver was finely minced, using aseptic precautions, and 10 grammes were weighed out in every case for the purposes of the experiment. To this were added 50 c.c. of sterile distilled water and a few drops of toluol. The substance to be tested was added to one, and the control and the one containing selenite were placed in an incubator at 38° C. for varying periods of time. They were then boiled and filtered. The filtrate and solid matter were each separately estimated by Kjeldahl's method.

SELENATE OF SODIUM			Soluble Nitrogen		Insoluble Nitrogen
Normal	...	...	0·0883	...	0·2125
Selenate 0·5 gramme		...	0·1076	...	0·1794
Normal	...	...	0·1674	...	0·1618
Selenate ½ %	...	...	0·1268	...	0·2047
Selenate 1 %	...	...	0·1570	...	0·1751

We see that there is no definite effect on autolysis caused by selenate of sodium.

SELENITE OF SODIUM

Third day

		Soluble Nitrogen		Insoluble Nitrogen
Normal boiled	... ...	0·0192	...	0·2711
Selenite 0·5 gramme	...	0·0211	...	0·2708
Normal liver	... ...	0·0883	...	0·2123

Seventh day

		Soluble Nitrogen		Insoluble Nitrogen
Normal liver	... ...	0·1911	...	0·1497
Selenite 0·5 grammes	...	0·0762	...	0·2636
Selenite 1 gramme ...	...	0·0566	...	0·2763

Tenth day

		Soluble Nitrogen		Insoluble Nitrogen
Normal liver	... ...	0·1674	...	0·1616
Selenite 0·5 grammes	...	0·0636	...	0·1171
Selenite 2 grammes ...	...	0·0745	...	0·1047

It is evident here that selenite of sodium has a very marked inhibitory effect on autolysis. The presence of selenite in a cell in sufficient amount would seem to inhibit all metabolic changes and destroy the cell. As the action of selenate of sodium in the body is similar to selenite in its ulterior effects, and as selenate is not poisonous to the cells, it seems evident that its toxic effects are present only when it has been reduced in the body to selenite.

The ease with which the glucose molecule is broken up by sodium selenite and selenate in the body suggested that if *diabetes mellitus* were due to the inability of the animal cells to break up the glucose molecule, as the believers in the oxidation theory hold, then the presence of selenite or selenate which effects this splitting up should lessen the amount of sugar in the urine, for it is well known that the degradation products of glucose can be easily dealt with by the diabetic. Selenate of sodium was the salt used, being less easily reduced in the intestine than the selenite. The patient was under the care of Dr. J. Hill Abram, whom I have to thank for trying the drug, and also Dr. A. F. Jackson for the care with which he performed the sugar estimations. The patient was a case of severe diabetes with twelve months' history.

			Per diem	
			Average sugar, in grains	Urea, in grains
Common diet	...	...	5232	538
Special diet	...	...	2212	307
Selenate	...	...	3087	455

The dose of selenate given was 5 minims of 1 per cent. solution, gradually increased until 25 minims was given.

As will be seen, there was an actual increase in excreted sugar while taking selenate, which seems to show that diabetes is not due to any lack of oxidation power nor to any difficulty in breaking up the glucose molecule.

Conclusions

These observations make it probable that selenate is reduced in the body to selenite, so that the action of selenite only need be considered.

When an injection of selenite is given, it is quickly taken up by the blood stream; only a small quantity is excreted by the kidney, the remainder is carried to the spleen and liver, where it is reduced by glucose to selenium. According to Zsigmondy, this reduction with glucose can, with the aid of the ultra microscope, be seen to take place fairly easily outside the body, particles of the reduced selenium being visible in about two minutes, so that the living cells would have no difficulty in effecting the same.

This reduction does not appear due to the aldehyde group of the sugar, but more probably is due to some special configuration of the glucose molecule, in which it is closely resembled by arabinose and levolose. This glucose, as required, is furnished from the glycogen of the liver, but when this is becoming exhausted fat is called upon. Whether the fat is used up as such or is transferred into sugar, it is difficult to say. The excretion of excess of fatty acid after an injection when sugar is urgently required, would point to the glycerine being possibly required for transference to sugar, but it is clear that there is no effort on the part of the organism to form sugar from proteins. If it were possible an effort would be made by the cells to manufacture glucose from protein to save themselves from destruction, but that no such action occurs is shown by the excretion of nitrogen remaining low, even until death. One must conclude that the organism cannot under such conditions transfer protein into sugar.

The evidence as to the transference or at least equivalence of fat and sugar is better. There is complete disappearance of fat as well as glycogen and sugar, which points to their transference or utilization. The loss of weight also suggests the same. In a moderately fat cat the loss of weight was about 38 per cent. This would just about represent the weight of fat, glycogen and glucose. Möchel's estimate of fat in a

moderately fat dog being 26 per cent., the remaining 12 per cent. would account for the glycogen and glucose.

It is necessary to consider at this stage the sudden disappearance of hydrochloric acid, the holding back of the chlorides by the organism, and the extraordinary relish for sodium chloride. These factors are all present when selenite has just been given, and when there is a sudden demand for glucose on the part of the cells. The most likely explanation is to be found in an observation by Eckhard that a one per cent. solution of common salt introduced into the blood caused glycosuria. Fisher showed that other sodium salts had the same effect, and that the stronger the salt solution the more glycosuria resulted, even up to 7·3 per cent. of sugar was found.

Excess of sugar in the urine represents excess of sugar in the blood, and therefore it would be possible for an animal holding back its chlorides by using its hydrochloric acid to fix some sodium salt, and taking sodium chloride in its food to raise its blood sugar content. If this sugar is picked out by the spleen and liver, and also possibly by leucocytes, it must result in a wonderfully increased power of reducing selenite to selenium, and so saving the cells from the poisoning effect.

If the organism is unable to neutralize the selenite, either because the selenite is in too great excess or because the available stores of glucose are used up, then selenite will act on and destroy the cells. The action is on the ferments of the cells, causing all metabolic changes to cease. It is curious to note here what a slight effect it has on bacteria and a powerful effect on ferments, quite a contrary effect to toluol, chloroform and similar antiseptics. It seems to show that the defences in single cells are much more highly developed than is the case in cells which rely on others for protection.

It is interesting to consider here whether glucose may not possibly be the means by which all reduction processes take place in the body. The well-known reductions taking place in the organism, such as methylene blue and Prussian blue, can be accomplished with ease by glucose in faintly alkaline solution. Considering the universality of occurrence of glucose in the body cells, and its well-known power of reduction outside the body, which is immensely multiplied within by cell activity, it would hardly seem necessary for the cells to require any other means of reduction.

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